METHODS AND COMPOSITIONS FOR SYNTHETIC RNA ENDONUCLEASES

STATEMENT OF PRIORITY

[0001] This application is a continuation application of, and claims priority to, U.S. application Ser. No. 16/021,892, filed Jun. 28, 2018 (allowed), which is a continuation application of U.S. application Ser. No. 15/602,546 (abandoned), filed May 23, 2017, which is a continuation application of U.S. application Ser. No. 15/353,485, filed Nov. 16, 2016 (abandoned), which is a divisional application of, and claims priority to, U.S. application Ser. No. 13/805,240, filed Jan. 31, 2013, and issued on Nov. 22, 2016 as U.S. Pat. No. 9,499,805, which is a 35 U.S.C. § 371 national phase application of Application Serial No. PCT/US2011/040933, filed Jun. 17, 2011, which claims the benefit, under 35 U.S.C. § 119(e), of U.S. Provisional Application Ser. No. 61/356,340, filed Jun. 18, 2010, the entire contents of each of which are incorporated herein by reference.

STATEMENT REGARDING ELECTRONIC FILING OF A SEQUENCE LISTING

[0002] A Sequence Listing in ASCII text format, submitted under 37 C.F.R. § 1.821, entitled 5470-561TSDVCT2DV_ST25.txt, 139,804 bytes in size, generated on Sep. 23, 2020 and filed via EFS-Web, is provided in lieu of a paper copy. This Sequence Listing is hereby incorporated by reference into the specification for its disclosures.

FIELD OF THE INVENTION

[0003] The present invention is directed to sequence specific restriction enzymes for site-specific cleavage of RNA, as well as methods of their use.

BACKGROUND OF THE INVENTION

[0004] Ribonucleases play important roles in various pathways of nucleic acid metabolism, including control of gene expression, mRNA surveillance and degradation and host defense mechanism against RNA viruses (1-3). Since the first ribonuclease was discovered as a heat stable enzyme from pancreas capable of digesting yeast RNA, a diverse panel of RNases has been characterized. However, unlike DNA restriction enzymes, a protein enzyme that cleaves RNA in a sequence-specific manner has not been found in nature. The known RNA endonucleases either specifically cleave their target through recognition of certain structures (e.g., RNase III family, RNase H or most ribozymes) (4-6), or have essentially no sequence specificity (e.g., RNase A cleaves after pyrimidine residues and RNase T1 cleaves after G residues) (7). The sequence specific cleavage of RNA can be achieved by a large multi-component complex such as the spliceosome or the RISC complex in RNAi pathway, each of which require guide RNA to recognize their targets (8, 9) and involve large protein/RNA assemblies, limiting their application in probing structured RNA or manipulating recombinant RNA in vitro.

[0005] Sequence specific cleavage of RNA has been achieved using engineered hammerhead ribozymes or RNA-cleaving DNAzymes (10). Both types of enzyme recognize their substrates through Watson-Crick binding arms of 6-12 nt, and therefore can achieve high target selectivity. However, these nucleic acid enzymes generally have low turnover rate (with k_{cat} around 1 min^{-1}) compared to protein enzymes, possibly due to tight binding to their substrates. In addition, the in vitro application of such nucleic acid enzymes is compromised by the high production cost and low stability of RNA, as well as the difficulty in controlling the folding of single stranded RNA or DNA.

[0006] The present invention overcomes previous shortcoming in the art by providing site specific RNA endonucleases and methods of their use.

SUMMARY OF THE INVENTION

[0007] In one aspect, the present invention provides a synthetic RNA endonuclease comprising the formula: A-B-C, wherein: A is an RNA binding domain, B is a linker peptide, and C is a cleavage domain.

[0008] In addition, the present invention provides a method of detecting a target RNA in a sample, comprising: a) contacting the sample with the RNA endonuclease of this invention under conditions whereby cleavage of RNA occurs if the target RNA is present in the sample and wherein the RNA binding domain of the RNA endonuclease is modified to bind the target RNA; and b) detecting a cleavage product of the target RNA, thereby detecting the target RNA in the sample.

[0009] Furthermore, the present invention provides a method of cleaving a target mRNA in a sample, comprising contacting the sample with the RNA endonuclease of this invention under conditions whereby cleavage of the target mRNA occurs and wherein the RNA binding domain of the RNA endonuclease is modified to bind the target mRNA, thereby cleaving the target mRNA in the sample.

[0010] In yet further aspects of this invention, a method is provided of cleaving a target mRNA in a cell, comprising introducing into the cell the RNA endonuclease of this invention, wherein the RNA binding domain of the RNA endonuclease is modified to bind the target mRNA, under conditions whereby cleavage of the mRNA occurs, thereby cleaving the target mRNA in the cell.

[0011] Also provided herein is a method of inhibiting expression of a target gene in a cell, comprising introducing into the cell the RNA endonuclease this invention, wherein the RNA binding domain of the RNA endonuclease is modified to bind mRNA encoding a gene product of the target gene, under conditions whereby cleavage of the mRNA occurs, thereby inhibiting expression of the target gene in the cell.

[0012] The present invention additionally provides a method of cleaving a target mRNA in a mitochondrion in a cell, comprising introducing into the cell the RNA endonuclease of this invention, wherein the RNA binding domain of the RNA endonuclease is modified to bind the target mRNA in the mitochondrion and wherein the RNA endonuclease comprises a mitochondrial targeting signal sequence, under conditions whereby cleavage of the target mRNA in the mitochondrion occurs, thereby cleaving the target mRNA in the mitochondrion in the cell.

[0013] Further provided herein is a method of inhibiting expression of a target mitochondrial gene in a cell, comprising introducing into the cell the RNA endonuclease of this invention, wherein the RNA binding domain of the RNA endonuclease is modified to bind mRNA encoding a gene product of the target mitochondrial gene and wherein the RNA endonuclease comprises a mitochondrial targeting signal sequence, under conditions whereby cleavage of the